

CLAIMS

What is claimed is:

- 1 1. A method for detecting the presence of at least one selected strain of an
2 organism in a sample, comprising the steps of:
3 providing a sample that may comprise nucleic acid from at least one selected
4 strain of an organism and nucleic acid from at least one non-selected strain of the
5 organism;
6 providing a plurality of primers substantially complementary to regions of
7 both said nucleic acid from at least one selected strain of the organism and said
8 nucleic acid from at least one non-selected strain of the organism;
9 exposing said sample to at least one probe that is sufficiently complementary
10 to a portion of said nucleic acid from at least one non-selected strain to block full
11 length amplification of said nucleic acid from at least one non-selected strain
12 between said plurality of primers, said at least one probe comprising a nucleic acid
13 analog;
14 amplifying said nucleic acid from at least one selected strain between said
15 plurality of primers; and
16 detecting amplification product of nucleic acid from at least one selected
17 strain.
- 1 2. The method of claim 1, wherein said at least one selected strain comprises a
2 pathogenic strain.
- 1 3. The method of claim 2, wherein said sample is derived from a subject and
2 said pathogenic strain indicates a risk of cancerous growth in said subject.
- 1 4. The method of claim 1, wherein said organism comprises human papilloma
2 virus (HPV).
- 1 5. The method of claim 1, wherein said at least one probe comprises PNA.

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1 6. The method of claim 5, wherein said at least one probe further comprises a
2 nucleotide different from PNA.

1 7. The method of claim 1, wherein each of said at least one probe comprises at
2 least 8 bases.

1 8. The method of claim 1, wherein the step of amplifying said nucleic acid of at
2 least one selected strain between said plurality of primers comprises conducting a
3 reaction selected from the group consisting of a polymerase chain reaction, a ligase
4 chain reaction, a rolling circle replication, a branched chain amplification, a nucleic
5 acid based sequence amplification (NASBA), a Cleavase Fragment Length
6 Polymorphism, ICAN and RAM .

1 9. The method of claim 4, wherein said regions of both said nucleic acids are
2 parts of a region selected from the group consisting of L1, L2, E1, E6, and E7 region.

1 10. The method of claim 4, wherein said at least one non-selected strain equals
2 all the low-risk HPV strains known.

1 11. The method of claim 4, wherein said at least one non-selected strain is
2 selected from the group consisting of HPV strains 6, 11, 42, 43, and 44.

1 12. The method of claim 4, wherein said at least one selected strain comprises a
2 plurality of high-risk HPV strains.

1 13. The method of claim 4, wherein said plurality of primers comprise MY09 and
2 MY11 (SEQ. ID. NOS. 10 and 11).

1 14. The method of claim 4, wherein said at least one probe is selected from the
2 group of sequences consisting of SEQ. ID. NO. 6 and SEQ. ID. NO. 7.

1 15. The method of claim 1, wherein said sample is a cervical scraping.

1 16. The method of claim 1, wherein said step of detecting amplification product
2 comprises in-gel electrophoresis of said product and staining said product with
3 ethidium bromide.

1 17. A method for detecting the presence of a target nucleic acid of a human
2 papilloma virus (HPV) in a sample cell, comprising the steps of:
3 suspending a sample cell in a solution;
4 isolating a target nucleic acid of a HPV from said sample cell;
5 contacting said target nucleic acid with at least one probe comprising peptide nucleic
6 acid (PNA), said at least one probe being substantially complementary to portions of
7 nucleic acids of multiple HPV types; and
8 detecting hybridization between said at least one probe and said target nucleic
9 acid.

1 18. The method of claim 1, wherein said solution contains an alcohol in an
2 amount sufficient to fix sample cells without coagulation, an anti-clumping agent,
3 and a buffer agent that maintains the solution at a pH within a range of about 4 to
4 about 7.

1 19. The method of claim 1, wherein said sample cells come from a subject and
2 wherein the presence of said target nucleic acid sequence indicates a risk of tumor
3 growth in said subject.

1 20. The method of claim 4, wherein said tumorous growth is associated with
2 either cervical cancer or endocervical carcinoma.

1 21. The method of claim 3, wherein the presence of said target nucleic acid
2 sequence is indicative of the presence of a particular type of HPV.

1 22. The method of claim 7, wherein said particular type of HPV is selected from
2 the group consisting of types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 70.

1 23. The method of claim 3, wherein absence of said target nucleic acid sequence
2 is diagnostic of absence of infection by HPV.

1 24. The method of claim 3, wherein absence of said target nucleic acid sequence
2 is diagnostic of absence of infection by HPV types selected from the group
3 consisting of types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 70.

1 25. The method of claim 1, wherein absence of said target nucleic acid sequence
2 is diagnostic of absence of infection by high-risk types of HPV.

1 26. The method of claim 1, further comprising amplification of said target
2 nucleic acid.

1 27. The method of claim 12, wherein said amplification step comprises
2 conducting a polymerase chain reaction.

1 28. The method of claim 1, further comprising capturing said target nucleic acid
2 onto a solid support through PNA-DNA interaction.

1 29. The method of claim 1, wherein each of said at least one probe comprises at
2 least 8 bases.

1 30. The method of claim 1, wherein said at least one probe comprises a
2 nucleotide different from PNA.

1 31. The method of claim 1, wherein said at least one probe is selected from the
2 group consisting of SEQ. ID. NOS. 1-5.

1 32. The method of claim 1, wherein said at least one probe is labeled with a
2 detectable marker.

1 33. The method of claim 17 wherein said at least one probe comprises a
2 molecular beacon probe.

1 34. The method of claim 1, further comprising using an antibody to recognize
2 said hybridization.

1 35. A method for detecting the presence of a target nucleic acid of a human
2 papilloma virus (HPV) in a sample, comprising the steps of:
3 capturing candidate nucleic acids that include a target nucleic acid on a solid
4 support;
5 contacting said candidate nucleic acids with at least one probe comprising
6 peptide nucleic acid (PNA), said at least one probe being substantially
7 complementary to portions of nucleic acids of multiple HPV types; and
8 detecting hybridization between said at least one probe and a target nucleic
9 acid.

1 36. The method of claim 20, wherein capturing candidate nucleic acids
2 comprises DNA-DNA interaction.

1 37. A method for in situ detection of the presence of a target nucleic acid of a
2 human papilloma virus (HPV) in a sample, comprising the steps of:
3 transferring suspended sample cells uniformly onto a surface;
4 in situ hybridizing a target nucleic acid of a HPV contained in said cells with at
5 least one probe comprising peptide nucleic acid (PNA), said at least one probe being
6 substantially complementary to portions of nucleic acids of multiple HPV types; and
7 detecting hybridization between said at least one probe and a target nucleic
8 acid.

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